response enhances mitochondrial metabolism triggered mitochondrial hyperpolarization and enhanced ATP production. Li^+ a substrate cation of NCLX but not of NCX replace Na^+ in enhancing the cytosolic and mitochondrial Ca^{2+} responses Altogether, our results show that combined electrical and ion flux activity of TTX sensitive Na^+ channels initiates a cytosolic Na^+ and Ca^{2+} signals propagating by the MCU and NCLX to the mitochondria, thereby shaping cytosolic or mitochondrial Ca^{2+} transients and metabolism of beta cells.

1225-Plat

The Potential for Another Calcium Uptake Mode in Cardiac Mitochondria

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Cytosolic Ca^{2+} levels are tightly regulated in cardiomyoctes. In many instances mitochondria play a direct role in this regulation. They take up Ca^{2+} via the Ca^{2+} uniporter, buffer Ca^{2+} by calcium-phosphate sequestration, and release Ca^{2+} primarily through the electrogenic $Na+/Ca^{2+}$ exchanger. However, the manner in which Ca^{2+} is taken up is still in dispute. In this study we used energized mitochondria isolated from guinea pig hearts to explore the potential for two modes of Ca^{2+} uptake. In addition, we sought to demonstrate the manner in which mitochondria take up, sequester, and release Ca^{2+}, and how Ca^{2+} transport is differentially modulated by Mg^{2+}. To carry out this study, we monitored extra-matrix and matrix [Ca^{2+}] during Ca^{2+} loading and unloading experiments using Fura-4F PP and Fura-4F AM fluorescence to quantify extra-matrix and matrix [Ca^{2+}], respectively. Two loading protocols were used: 1) a bolus of CaCl2 was added to a suspension of mitochondria in respiration buffer and 2) mitochondria were added to respiration buffer already containing CaCl2. In all experiments, ruthenium red was later added to stop Ca^{2+} uptake and NaCl was added to initiate Ca^{2+} efflux. Also, each protocol was executed in the presence and absence of extra-matrix MgCl2. Depending on the protocol, two distinct profiles of Ca^{2+} uptake were observed, whereby using protocol 1 resulted in a faster mode of Ca^{2+} uptake and protocol 2 in a slower mode. Furthermore, Ca^{2+} uptake and efflux were inhibited by MgCl2. We found that Mg^{2+} reduced the ability of mitochondria to sequester Ca^{2+} independent of the protocol. In summary, these observations derived from our experiments show the potential for at least two modes of Ca^{2+} uptake and provide us with a better understanding of how matrix Ca^{2+} dynamics change under physiological and pathophysiological conditions.

1226-Plat

Ultrafast Genetically Encoded Calcium Indicators for Visualizing Calcium Flux and Action Potentials

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Genetically encoded calmodulin-based calcium probes (GCaMPs) have become the reporters of choice for visualising the calcium flux associated with action potentials *in vivo*. A major limitation of currently available GCaMPs is the slow kinetics of fluorescence changes induced by calcium association and dissociation. We have addressed this issue by generating a series of mutants of GCaMP3 in the calcium binding sites of calmodulin alone[1] and in combination with mutations in the RS20 target peptide sequence[2] with the view of lowering the affinity for calcium and accelerating the calcium response kinetics. The calcium association kinetics for the resulting GCaMP3 EF-hand and peptide mutants were highly cooperative and characterized by a rate limiting conformational change. Fluorescence changes on calcium association were up to 7-fold faster compared to GCaMP3. Calcium dissociation rates were up to 60-fold faster than GCaMP3 and 25-fold faster than the newly developed GCaMP6 fast (GCaMP6f). Dissociation constants (K_d) for calcium were in the μM range with Hill coefficients from 2 to 5. Two-photon cross-sections of mutants were comparable to GCaMP3. Fluorescence responses of mutated GCaMP3s to calcium transients in endothelial cells were similar to those seen with small molecule indicators. The principles employed proved to accelerate the calcium kinetics of GCaMP3 and can be applied to the new generations of GCaMPs to generate low affinity probes.

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1227-Plat

A New Ca^{2+} Probe, Calstabi-Cam, Targeted to Ryanodine Receptors of Cardiomyocytes

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The contractile force of cardiomyocytes is controlled by Ca^{2+} cross-signaling between L-type Ca^{2+} channels and ryanodine receptors (RyR2) across the narrow dyadic cleft. To detect the junctional Ca^{2+} signal, we designed a peptide probe (Calstabi-Cam) with calmodulin as its Ca^{2+} sensor, yellow fluorescent protein (EYFP) as reporting fluorophor, and FKBP12.6 (calstabin2) as subunit of RyR2. Effective adenoviral expression in cultured adult rat cardiomyocytes was verified after 48 hours when Calstabi-Cam co-localized with fluorescent RyR antibodies in a sarcomeric z-lines pattern. Dissociation constant (k_d) of Calstabi-Cam for Ca^{2+} measured in permeablized myocytes was 80 nM. The kinetic of Ca^{2+} signals was measured in voltage-clamped cells with a Leica TIRF microscope which allowed comparison of rapidly interlaced images of cytosolic Ca^{2+} probes (fura-2 or fluo-4) and Calstabi-Cam. Fluo-4 Ca^{2+} sparks were detected superimposed on the sarcomeric fluorescence patterns of Calstabi-Cam. On activation of Ca^{2+} release by caffeine or membrane depolarization, Calstabi-Cam fluorescence signals had slower rise times compared to fura-2, but had much slower decay kinetics. Scans of focal Calstabi-Cam signals at different sites, occurring spontaneously or at the onset of evoked Ca^{2+} releases, appeared to have a significant distribution of magnitudes and latencies. We conclude that Ca^{2+}-sensing biological peptides may be targeted to the cleft-space occupied by DHPR/RyRs complex as to make it possible to record the variance of Ca^{2+} signals at different dyadic junctions.

1228-Plat

CaMKII-Mediated Amplification is Essential to NAADP Signalling in Cardiac Myocytes

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NAADP is a highly potent endogenous Ca^{2+}-mobilising second-messenger forming part of the beta-adrenergic response in cardiac myocytes[12]. Our previous work suggests NAADP causes Ca^{2+}-release from acidic endolysosomal stores, leading to additional uptake by the SR[12]. Questions have arisen regarding whether the magnitude NAADP-mediated responses can be accounted for by acidic-store-mediated Ca^{2+} release through TPC channels[34]. This study aimed to confirm that TPC2 channels are required for NAADP responses in cardiac myocytes and investigate the possibility of amplification in the pathway.

Transgenic mice were utilised to investigate the role of TPCs. Rapid application of NAADP-AM to WT murine ventricular myocytes elicited a significant increase in calcium transient amplitude ($16 \pm 5\%$, $P<0.05$). This response was abolished in cells isolated from TPC2KO mice ($-6 \pm 4\%$, $P>0.05$).

NAADP photorelease in guinea pig atrial or ventricular myocytes caused a significant increase in calcium transient amplitude (of $37 \pm 8\%$ and $38 \pm 9\%$ respectively, both $P<0.05$), accompanied by acceleration in the rate of calcium transient decay (by $23 \pm 6\%$, atrial, and $28 \pm 9\%$, ventricular, myocytes, both $P<0.05$).

In the presence of KN93 (atrial myocytes) or AIP (ventricular myocytes), to inhibit cellular CaMKII function, no changes in Ca^{2+} transient amplitude or decay velocity were observed after NAADP photorelease ($P>0.05$, both measures, both cell types). Similarly, no changes in Ca^{2+} transients were observed during photorelease in the presence of the NAADP receptor antagonist, Ned-19 ($P>0.05$, both measures, both cell types).

These data support the hypothesis that NAADP-induced Ca^{2+} release requires TPC2, and suggest CaMKII is the major effector for its actions in cardiac myocytes.

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1229-Plat

Calcium Signaling Inside Cilia Upon Mechanical Bending

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The primary cilium is a sensory organelle central to many signaling pathways. Direct visualization of signaling dynamics within primary cilia constitutes a major technical challenge due to the sub-micron dimensions of the organelle as well as its close proximity to the cell body. By newly designing and developing a genetically encoded calcium indicator (GECI) targeted to primary cilia without compromising indicator efficiency, we now demonstrate the unprecedented capability to visualize Ca^{2+} dynamics within the ciliary lumen with high specificity, sensitivity and wide dynamic range. Simultaneous